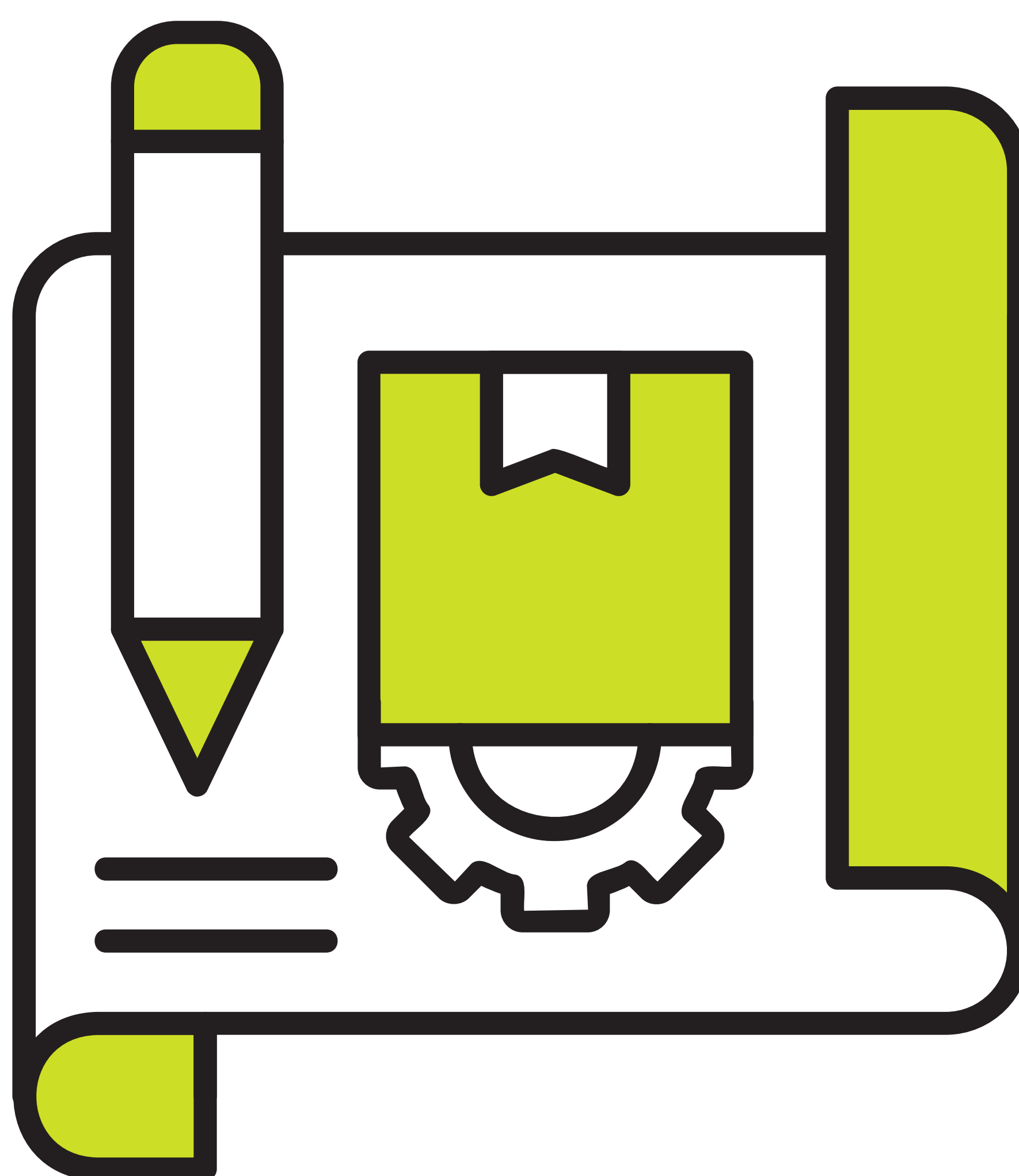




HoloRuminant
Understanding microbiomes of the ruminant holobiont

HOLORUMINANT PROTOCOL

Protocol for Milk Sampling



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Materials needed:

- ◇ Gloves
- ◇ Skin disinfectant (surgical spirit, ethanol or iodine)
- ◇ Towels
- ◇ Sterile 15ml tubes
- ◇ Cotton wool

Notes: Be sure not to overfill the tubes, as the milk will expand upon freezing.

1. Wear new gloves for each new animal.
2. Clean the udder with a clean towel to clear debris. When cleaning the udder, ensure gentle wiping with the towel to avoid irritation. If it's dirty, make sure the towel is clean and non-contaminated.
3. Hand strip and discard the first 6 squirts of milk, to avoid contamination of the sample with environmental microbes.
4. Soak a piece of cotton wool with skin disinfectant and clean the teat to sample. One teat will be sampled at a time. After cleaning one teat, discard the used cotton wool and repeat for the next teat
5. Open the sample bottle, keep the lid facing downwards and the opened bottle almost horizontal (prevents contamination with dust and bacteria).
6. With the bottle between horizontal and 45-degree angle, fill the 15ml tube and replace the cover immediately. Do not let the teat touch the bottle to avoid introducing contaminants. Additionally, ensure that milk does not touch your hands.
7. Label the transport tube, including animal identification number, type of sample, and date.
8. Snap-freeze in dry ice or liquid nitrogen, and later at -80°C.